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(54) Title: IMPROVED THERAPEUTIC FORMULATION AND METHOD

(57) Abstract

The invention provides a method of treatment or prophylaxis of disease in an animal, said method comprising administering effective amounts of substantially whole antibody and one of more strains of suitable probiotic organisms to said animal.

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IMPROVED THERAPEUTIC FORMULATION AND METHOD

This invention relates to a method and composition for treatment or prevention of disease.

Background and Prior Art

Gastrointestinal disease is a significant cause of morbidity and mortality in humans and in domestic animals, particularly in the first few weeks of life. A high proportion of hospital admissions of babies results from gastrointestinal infection, which leads to rapid dehydration, and may prove fatal. Among domestic animals, particularly in intensive rearing situations, gastrointestinal infection spreads extremely rapidly, and results in failure to thrive, often leading to death. The effects of these conditions are particularly devastating in the production of pigs, dairy calves and poultry.

While in human patients the treatment largely depends on oral or intravenous rehydration therapy, in the farm situation efforts to contain gastrointestinal infection have largely relied upon feeding of large amounts of antibiotics in either feed or water. This is very costly and suffers from the disadvantage that resistance of the causative organisms to the antibiotics is likely to arise, and to spread to other, possibly more dangerous organisms.

In most domestic animals, maternal antibodies are transferred to the progeny in colostrum. Vaccines able to stimulate high levels of maternal antibody against specific intestinal pathogens of neonatal animals are now widely used in intensive animal production. In situations where young animals are too weak to suckle or are not kept with their mothers, various artificial colostrum products have been used in an attempt to provide passive immunity. For example a product designed for small weak piglets named ReSus is manufactured by Nufarm Limited. This product combines hyperimmune colostrum from dairy cows vaccinated

against strains of *Escherichia coli* that infect neonatal piglets in a high energy food base. Other products, available overseas, use immunoglobulins obtained from colostrum, milk, whey or plasma.

In the case of rotavirus diarrhoea, it is known that the most important protective factor is the presence of specific antibody in the lumen of the small intestine. Protection against rotavirus diarrhoea can be achieved by oral administration of IgG, whether the IgG is homologous or heterologous (Snodgrass D.R. *et al*, Infect. Immun., 1977 16 268-270; Barnes, G.L. *et al*, Lancet 1982 1 1371-1373) or via colostrum of vaccinated cows (Mebus, C.A. *et al*, J. Am. Vet. Med. Assoc., 1973 163 880-883). It was subsequently shown that oral administration of bovine colostrum from immunized cows to human infants was effective in protection against rotavirus diarrhoea, (Hilpert H. *et al*, J. Infect. Dis., 1987 156 158-166; Davidson G.P. *et al*, Lancet 23 September 1989 709-712, Turner R.B. and Kelsey D.D. 1993, Pediatric Infectious Diseases J., 12:718-722).

Intact colostral antibody has been found efficacious in treatment of *Helicobacter pylori* infections, which may be associated with gastritis and peptic ulcer disease. This method is the subject of Australian Patent Application Number 80207/91 by Abbott Laboratories, entitled "Method for the treatment of gastric disease", the entire disclosure of which is herein incorporated by reference. Efficacy in this instance was obtained by regular ingestion of intact colostral whey antibody. This specification describes in detail methods for immunisation with *Helicobacter pylori*, and methods for isolation and concentration of specific antibodies from mammary secretions, including milk and colostral whey, of animals immunised with *Helicobacter pylori*, and in particular bovine colostral whey.

Methods for production of immunoglobulins with specificity against various organisms from lactating mammals are also disclosed in U.S. Patents No. 3128230 and

No. 4051231. Australian Patent Application No. 644468 (82527/91) discloses a process for preparation of a spray-dried colostrum product which can be applied to immune or hyperimmune colostrum, which is stated to be useful in the treatment or prevention of rotavirus infection in infants.

Hyperimmune colostral antibodies directed against organisms causing gastrointestinal disease have been widely recognised to be effective in disease control (Tackett *et al.*, New England J. Med. 1988 318 1240-1243; Hilpert *et al*, J. Infect. Dis., 1987 156-158; Ebina *et al*, Med. Microbiol. Immunol., 1985 174-177; Davidson *et al*, Lancet, 1989, 23 September 709-712).

Orally-administered protease has also been shown to influence the potential for microbial colonisation of the small intestine by degrading receptors for microbial adhesins and toxins (Mynott *et al*, Infection and Immunity, 1991 59 3708-3714, Chandler et al, 1994 Vet. Microbiol. 38:203-215).

Although it is well known that limited digestion of immunoglobulin molecules with proteolytic enzymes such as pepsin and papain cleaves the immunoglobulin to form Fc and either Fab or $Fab(_2)$ fragments, this is effected by limited digestion only, and must take place under controlled conditions. Unless the conditions are carefully controlled, some proteases will completely break down the antibody, and destroy its activity.

We have previously found that an improved response may be obtained by combining administration of protease-cleaved antibody in order to prevent or alleviate gastrointestinal disease (International Patent Application No. PCT/AU94/00121, Pharma Pacific Pty.Ltd.). Administration of protease-cleaved antibody was considered particularly beneficial in neonates, where gastric and intestinal proteolytic activity is low because of developmental immaturity, (Moughan P.J. *et al*, In. Nutritional Triggers for Health and in Disease; Simopoulos A.P. (ED) Worlds Rev. Nutr. Diet. Basel, Karger, 67 40-113)

Because of the apparent favouring of development of Gram positive flora (lactobacili, streptococci,or both) in the gastrointestinal tract (GIT) following treatment with pepsin-digested antibody, the previous invention proposed that an exogenous culture of these organisms, if administered concurrently with the protease-treated antibody, would have an improved chance of colonisation. Cultures of lactobacilli and streptococci are currently used commercially, with limited success, to control diarrhoeal diseases in piglets and other species. These cultures are called probiotics. The main problem with the therapeutic function of probiotics is the difficulty in establishing these strains in the gastrointestinal tract (Cain, C.,1988. Observations of indigenous and non-indigenous lactic acid bacteria as potential probiotic organisms in pigs. M.Sc. Thesis, School of Agriculture, La Trobe University).

The present invention provides a further and simpler means of improving the colonisation of the intestinal tract by probiotic strains, thereby extending the effectiveness and/or period of disease protection offered by oral administration of antibody. It also allows antibiotic use to be avoided where possible.

In work leading up to the invention the present inventors have surprisingly found that administration to young animals of antibody in a substantially whole form, together with a probiotic organism gives high levels of protection against gastrointestinal disease. This is surprising since it was previously thought that it was necessary to subject the antibody to proteolytic digestion in order to obtain efficient prophylaxis or treatment of a disease. In addition, the inventors have also surprisingly found that use of substantially whole antibody and a probiotic organism provides a synergistic effect in the treatment of gastrointestinal disease.

Summary of the Invention

In one aspect the invention provides a method of treatment or prophylaxis of disease in an animal said method comprising administering effective amounts of substantially whole antibody and one or more strains of suitable probiotic organisms to said animal, wherein said animal is not naturally protected by exposure to appropriate or adequate antibody.

The method encompasses both treatment and prevention of disease. The diseases which may be treated by the method are diseases of the alimentary tract, reproductive tract and include oral diseases, gastrointestinal diseases, vaginal diseases caused by the presence or colonisation of inappropriate or pathogenic microorganisms such as bacteria, viruses and fungi.

The term "animal" refers to any animal capable of treatment or prophylaxis by the method of invention such as mammals (Eg. humans, domestic animals such as pigs, cattle, sheep, goats, horses, llamas, alpacas) and other animals such as poultry for example, chickens, geese, ducks and larger birds such as emus and ostriches and the like.

The term "effective amounts" refers to the amounts of the antibody and one or more probiotic organisms required to effectively treat or prevent the disease.

The term "substantially whole antibody" refers to an antibody which has not been treated proteolytically or is not intentionally treated to degrade the antibody or to modify it to any degree. The antibody suitable for use in the invention does not have to be purified, and may be derived from immune serum or colostrum, from egg yolk of immunised poultry or may be a monoclonal antibody or a bioengineered antibody. The antibody of the invention is preferably an immunoprotective antibody which means that the antibody has specificity against the pathogenic agent of the disease targeted or against a toxin or other molecule produced by the pathogen which alone or in combination with a probiotic agent protects against the

pathogenic effects of the causative organism of the disease. Colostrum from immunised dairy animals, such as cows, sheep or goats, is especially convenient for use in the invention. The antibody may be IgG, IgA or IgM, but is most preferably bovine IgG_1 and/or IgA. Antibody from egg yolk (IgY) may also be used. It will be appreciated that one or more antibodies may be used.

The substantially whole antibody and one or more suitable probiotic organisms may be administered together, separately or sequentially to the animal. Most preferably the substantially whole antibody and one or more suitable probiotic organisms are administered together.

The term "suitable" in reference to the one or more strains of probiotic organisms means suitable for prophylaxis or treatment of the disease targeted. The causative organisms and associated toxins of such diseases which may be treated or prevented include, but are not limited to, *Escherichia coli, Clostridium difficile, Helicobacter pylori, Cryptosporidium* species, Mycobacterium species, Candida species, *Microsporidia* species such as *M.paratuberculosis* and rotaviruses.

The term "probiotic organism" refers to any microorganism capable of imparting a beneficial effect on the animal when administered orally or by another route to such animal. The probiotic organism is suitably an organism indigenous to the species of the animal to be treated, although it may be a non-indigenous member of the mucosal flora of healthy individuals of other species. Preferably the probiotic organism includes, but is not limited to, a *Lactobacillus, Bacillus, Streptococcus, Enterococcus* or a *Bifidobacterium*. A mixture of two or more probiotic organisms may be used. Probiotic strains may be selected on the basis of their ability to colonise effectively and to flourish on the infected surface, or potentially infected surface, to be treated. They may be strains that possess properties that are antagonistic toward the pathogen being displaced. Alternatively the

probiotic organism may permit selective overgrowth in the host thereby precluding or excluding the pathogen.

The term "wherein said animal is not naturally protected by exposure to an appropriate or adequate antibody" refers to an animal which is not receiving appropriate antibody or adequate levels of such antibody from drinking mother's milk and other situations where the animal is not ingesting or otherwise producing appropriate or adequate endogenous antibody, such as in the case where the animal's immune system is functioning below its normal abilities or where the onset of the disease is acute and the host's immune system has not recently been exposed to the pathogen, such as traveller's diarrhoea in humans.

The method of the invention may be used in conjunction with other treatments, such as antibiotic treatment, or electrolyte therapy. Preferably antibiotic treatment is avoided unless absolutely necessary, in which case a probiotic with appropriate antibiotic resistance should be used.

While the invention is specifically described with reference to gastrointestinal disease, it will be clearly understood that the invention is applicable to the treatment or prevention of disease at other sites which are subject to changes in flora during disease, such as the oral cavity and the vaginal tract. The mode of administration will be chosen depending on the site of intended treatment. Modes of administration contemplated include lozenges or other tablets, pessaries, creams, lotions, washes and so on.

The methods of the invention are suitable for treatment of immunocompromised patients or patients particularly prone to infection, such as HIV-infected patients or patients with symptoms of AIDS-related complex or AIDS, or patients suffering from extensive burns or scalds, or for the treatment of patients suffering from gastrointestinal malabsorption syndromes or inflammatory bowel disease.

The methods of the invention are also suitable for treatment of patients receiving H_2-receptor antagonists such as Zantac or Tagamet, or proton-pump inhibitors such as Losec, which inhibit acid secretion in the stomach, and have diarrhoea as a frequent side-effect.

In a second aspect, the invention provides a composition for treatment or prevention of gastrointestinal disease in an animal, comprising

a) an effective amount of substantially whole antibody, and

b) an effective amount of one or more strains of suitable probiotic organisms, together with a pharmaceutically-acceptable carrier.

In a preferred embodiment for administration to subjects suffering diarrhoeal disease, the formulation provides a multiple format, including antibody, probiotic and electrolytes useful to offset the losses induced by pathogen colonisation.

In an alternative embodiment, the formulation may comprise a multi-component tablet, optionally enteric coated or in the form of a suspension or granules for reconstitution, in which the probiotic and antibody are combined. Conventional fillers, granulating agents, excipients may be present. Variations will be obvious to the person skilled in the art.

The invention also provides formulations for use in the aforesaid method. Individual formulations will depend upon the antibody and probiotic type, and can be devised using known formulation principles and normal trial-and-error experimentation.

For application to particular sites, liquid formulations including drops or sprays, or aerosol formulations may be particularly suitable.

Detailed Description of the Invention

The invention will now be illustrated by way of reference only to the following non-limiting examples:

Example 1 **Use Of Antibody And Probiotic To Protect against Diarrhoeal Disease In Piglets**

This experiment was designed to investigate whether passive immune protection for piglets during challenge with a pathogenic ($K88^+$ *E. coli*) was best achieved using intact antibody contained in a high energy colostrum replacer specially formulated for use in piglets, or intact antibody purified from the same batch of colostrum, either given with or without a probiotic selected for use in piglets. $K88^+$ *E.coli* infection in piglets is typical of entero-toxigenic *E.coli* infections of other monogastric animals, including many types of traveller's diarrhoea in humans.

Treatment and dosage regimen

Treatments consisted of six twenty ml doses given to piglets at approximately 6h intervals. Piglets were taken from the sow at birth (before suckling from the sow) weighed and ear-tagged. Approximately weight-matched groups of piglets were allocated randomly to one of six following treatment groups as shown in Table 1.

Table 1. Treatment group description.

Abbreviated Designation	Treatment Description
Control	Milk replacer given in place of treatments
Res	Colostrum Replacer (ReSus, Nufarm Animal Health). Approx. 12.5mg/ml bovine IgG
Ab	Purified colostral antibody in milk replacer. Approx. 12.5mg/ml bovine IgG
Prob	Lactobacillus fermentum probiotic. (approx. 10^9cfu/ml milk replacer)
AbProb.	Combined probiotic and purified colostral antibody treatment
ResProb	Combined probiotic and ReSus treatment

Piglets in the colostrum replacer-treated groups received a commercial colostrum replacer designated as ReSus (Nufarm Animal Health). ReSus contains bovine colostrum from cows immunised against the *E. coli* types which infect piglets, using polyvalent whole cell and pilus-based vaccines. Piglets in the antibody treatment groups received bovine colostral antibody from the same bulk batch of colostrum used in the manufacture of the ReSus batch referred to above, but in this case the antibody had been removed from the base colostrum by fat removal and acid precipitation of the casein. The antibody was then further purified by $(NH_4)_2SO_4$ precipitation and dialysis against distilled water. (Fang, W.D. and Mukkur, T.K.S., Biochem. J., 1976 155 25). Antibody-containing treatments were diluted with milk prior to use, such that they have an equivalent titre of blocking activity in an ELISA blocking assay to that of ReSus. The blocking assay consisted of $K88^+$ *E. coli* on the solid phase, followed by test antibody, anti-K88 enzyme conjugate and enzyme substrate.

Challenge doses consisted of a sheep blood agar lawn culture of haemolytic $K88^+$ *E. coli* (strain WG, O149;K91;K88ac;H10, Tzipori *et al*, Aust. Vet. J., 1980 56 274) suspended in 2ml of sterile phosphate-buffered saline (PBS, 0.1M, pH 7.2). This dose consisted of approximately 5×10^9 cfu/dose.

Probiotic treatments were prepared by suspending from Rogosa Agar a 48h anaerobic lawn culture of *Lactobacillus fermentum* (strain 104, kindly supplied by Dr. P. Conway, Biotechnology Department, University of New South Wales) into each 20ml treatment dose of combined probiotic:antibody (ReSus or purified antibody), or milk replacer for piglets in the probiotic only treatment group. Control piglets were given the same volume of commercial milk replacer at the same regimen as piglets given the probiotic and antibody-containing treatments. Treatment doses, milk feeds and bacterial challenge doses were given

by oro-gastric tube.

Piglet Management

Piglets (56) born to six sows were used in the trials. After allocation to treatment groups piglets were transferred to heated cages, usually as pairs. Where bullying was a problem or odd numbers were available in the treatment group, piglets were housed individually. Piglets receiving treatments containing probiotic were housed and treated using facilities located on opposite sides of the cage room. At about two hours after birth the piglets were given their first treatment dose, followed thirty minutes later by a bacterial challenge dose.

A second similar challenge dose was given 24h later. All piglets were killed with an overdose of barbiturate at about 36h after birth, or earlier if debilitated by disease.

Microbiological Assessments

Immediately after death intestinal scrapings were taken from the stomach and three sites in the small intestine (SI). The stomach was sampled half way around the greater curvature, while the small intestine was sampled 200mm from either end and half-way between. These sites were designated site 1 (duodenal end), site 3 and site 2, respectively. Scrapings from $1cm^2$ of mucosa at each site were suspended in sterile Peptone Water (1.0ml, 0.1% w/v Oxoid). Bacterial counts were then performed according to the method of Miles and Misra (1932) using Sheep Blood Agar, MacConkey Agar and Trypticase Soya Agar (TSA) incubated aerobically overnight at 37°C, and Rogosa Agar incubated anaerobically for 48h at 37°C. Counts were made of haemolytic (Hly^+) colonies $\geq$ 1mm diameter on the blood agar. Some of these colonies were confirmed by slide agglutination to resemble the challenge strain. Estimates of coliform numbers (both lactose fermenting and non-fermenting) were made from MacConkey Agar, and

lactobacillus from the Rogosa Agar. TSA was used to assess total numbers of aerobic bacteria.

Condition and faecal scores

Scores were estimated prior to administration of treatments. Where necessary rectal swabs were taken to facilitate faecal scoring. Piglets were assessed according to the following scales. Where doubt existed as to correct placement, intermediate (half) scores were given.

Condition scores

0. Pig normal, bright temperament
1. Piglet slightly depressed. Approximately one second delay in skin return on pinch test
2. Piglet depressed. One to three second delay on pinch test.
   Eyes dull, dry appearance to mucosal surfaces.
3. Obvious depression and dehydration. Greater than three second delay on pinch test.
4. Piglet in poor condition and removed from trial.

Faecal scores

0. Normal soft damp to formed faeces or meconium.
1. Faeces of greater volume and/or more moist consistency than normal. Unformed.
2. Semi-liquid faeces with uncontrolled passage on stimulation. Dehydration symptoms not obvious.
3. Semi-liquid or liquid faeces with symptoms of dehydration.
4. Liquid faeces with appearance of prolonged scouring. Dehydration obvious.

Statistical Analysis

The analysis included blocking for farrowing date. Since not all treatments were included at each date, the design was unbalanced. We used residual Maximum Likelihood (REML) to determine the significance of

treatment differences using the statistical package GENSTAT 3.1, Lawes Agricultural Trust (Rothamsted Experimental Station).

Analyses of the bacterial numbers in the intestines of piglets treated prophylactically (prior to and subsequent to bacterial challenge) are shown in Tables 2 - 5.

Table 2. Mean haemolytic (Hly^+) *E.coli* numbers ($1og_{10}$ transformed) in the GIT of challenged piglets.

Treatment	Stomach	SI - 1	SI - 2	SI - e3
Control	7.489^{a*}	8.192^{a}	8.043^{a}	8.702^{a}
Ab	5.584^{b}	6.277^{b}	6.076^{b}	7.069^{b}
Res	5.598^{b}	6.124^{b}	5.727^{b}	6.431^{b}
Prob	6.589^{ab}	6.128^{b}	6.089^{b}	6.608^{b}
AbProb	5.225^{b}	5.356^{b}	4.820^{b}	6.523^{b}
ResProb	5.551^{b}	5.551^{b}	5.134^{b}	6.726^{b}
LSD 5%	1.502	1.347	1.487	1.608

* Means with any similar superscript are not significantly different ($p>0.05$).

"SI" used above means small intestine; 1 means region 1, 2 means region 2 and e3 means region 3.

"LSD" means least significant difference.

Table 3. Mean lactobacillus numbers (log_{10} transformed) in the GIT of challenged piglets.

Treatment	Stomach	SI - 1	SI - 2	SI - 3
Control	5.934^{ab*}	5.572^{a}	5.414^{ab}	5.134^{bc}
Ab	5.523^{b}	5.238^{a}	4.358^{b}	4.761^{b}
Res	5.366^{b}	4.869^{b}	5.064^{ab}	4.384^{b}
Prob	6.820^{a}	6.193^{a}	5.890^{a}	6.584^{a}
AbProb	6.294^{ab}	5.889^{ab}	5.960^{a}	5.842^{ac}
ResProb	7.021^{a}	6.318^{a}	6.152^{a}	6.903^{a}
LSD 5%	1.126	1.162	1.119	1.216

* Means with any similar superscript are not significantly different ($p>0.05$)

Table 4. Mean coliform numbers (log_{10} transformed) in the GIT of challenged piglets.

Treatment	Stomach	SI - 1	SI - 2	SI - 3
Control	6.973^{ab*}	7.416^{a}	7.673^{a}	7.961^{a}
Ab	5.382^{ab}	6.386^{ac}	6.892^{a}	7.438^{a}
Res	6.536^{a}	6.504^{ac}	6.527^{ac}	6.322^{a}
Prob	5.700^{ab}	5.371^{b}	5.805^{bc}	6.494^{a}
AbProb	5.590^{b}	5.422^{bc}	5.539^{bc}	6.939^{a}
ResProb	5.397^{b}	5.797^{bc}	5.389^{b}	6.775^{a}
LSD 5%	1.270	1.104	1.309	1.644

* Means with any similar superscript are not significantly different ($p>0.05$)

Table 5. Mean bacterial numbers (log_{10} transformed) over all GIT sites.

Treatment	Hly+ *E.coli*	Lactobacillus	Coliform	Total Aerobe
Control	8.115[a*]	5.509[b]	7.511[a]	7.984[a]
Ab	6.228[b]	4.959[b]	6.507[ac]	6.976[b]
Res	5.975[b]	4.923[b]	6.460b[c]	6.651[b]
Prob	6.360[b]	6.373[a]	5.832b[c]	6.548[b]
AbProb	5.463[b]	6.005[a]	5.866b[c]	6.495[b]
ResProb	5.670[b]	6.585[a]	5.828b[c]	6.650[b]

* Means with any similar superscript are not significantly different ($p>0.05$)

Total lactobacillus numbers in control pigs were higher in these experiments than those in antibody-treated piglets. Colonies formed by bacteria in these counts however generally did not resemble those seen in probiotic-treated piglets. The higher count appeared to be a function of the generally higher bacterial contamination in the intestines of the debilitated untreated piglets, rather than cross-contamination between treatment groups. Even if cross-contamination did occur, this does not detract from the significance of our results.

As expected, lactobacillus colonisation was highest in the probiotic treated groups. When colonisation was expressed as a ratio of Hly+ *E.coli*:lactobacillus numbers (undesirable:desirable flora), a significantly ($P<0.05$) more favourable ratio was obtained with the combined antibiotic:probiotic therapies over the antibody-only treatments at the stomach upper and mid-small intestine sites, and for the overall mean count ratio. (Table 6). Bacterial infection of the mid and upper small intestine is most correlated to serious diarrhoeal disease (Moon H. W. *et al* 1979: Am. J. Clin. Nutr. 32 : 119-127).

Table 6. Ratio of Hly$^+$:lactobacillus numbers in piglet GITs.

Treatment	Stomach	SI - top	SI - mid	SI - end	Mean
Control	1.317^{c*}	1.647^{b}	1.653^{c}	1.919^{c}	1.596^{b}
Ab/Res	1.185^{bc}	1.384^{b}	1.420^{bc}	1.598^{bc}	1.362^{b}
Prob	0.996^{ab}	1.003^{a}	1.047^{ab}	1.016^{a}	1.000^{a}
Ab/ResProb	0.846^{a}	0.928^{a}	0.836^{a}	1.165^{ab}	0.913^{a}
LSD 5%	0.280	0.375	0.386	0.442	0.288

* Means with any similar superscript are not significantly different ($p>0.05$).

A significant difference between the combined antibody probiotic treatments and probiotic only treatments was not demonstrated, although a more favourable mean ratio was again obtained in all but the lower SI. The probiotic alone, however, did not significantly improve the Hly$^+$ *E.coli*:lactobacillus ratio over antibody-only treatments at the stomach or mid-SI sites.

A corresponding benefit in the combined antibody:probiotic therapy over probiotic (or antibody) therapies alone in terms of reducing condition and faecal (diarrhoea) scores was also observed. (Table 7).

Table 7. Mean condition scores of piglets recorded immediately prior to *post mortem* examination (T) and 12h prior to death (T-12h).

Treatment	Mean condition score T	Mean condition score T-12h
Control	3.21	1.17
Ab	1.79	0.58
Res	1.36	0.75
Prob	1.59	1.22
AbProb	0.75	0.23
ResProb	0.86	0.55

Table 8. Mean faecal scores of piglets recorded immediateley prior to *post mortem* examination (T) and 12h prior to death (T-12h).

Treatment	Mean faecal score T	Mean faecal score T-12h
Control	3.67	1.42
Ab	2.21	1
Res	2.08	1.22
Prob	2.38	2.44
AbProb	1.75	0.82
ResProb	0.86	1.06

The prior art teaches that antibody treatment alone is currently the most effective non-antibiotic therapy for gastrointestinal infections. This trial indicates that a probiotic well able to colonise the piglet intestines, was less able to control infection or disease than antibody alone.

The results of this trial also indicate however that intact antibody, in combination with an appropriate

probiotic, is able to better protect the intestine from attaining diarrhoea-producing levels of infection under severe challenge conditions. The combined therapy was able to provide a significant ($p>0.05$) improvement in the ratio of undesirable to desirable organisms in the intestine over antibody alone. The combination therapy gave demonstrable benefits over either antibody treatment or probiotic treatment alone.

Example 2. Use of Antibody and Probiotic to Treat Diarrhoeal Disease in Piglets.

This experiment was designed to determine whether the high level of protection against bacterial challenge provided by combined antibody:probiotic treatments in the previous experiment could be extended to improved treatment of pre-existing diarrhoeal disease.

Treatment and dosage regimen

Colostrum-deprived piglets were allocated to six treatment groups, as outlined for Example 1. In this experiment piglets were given their first challenge dose at 2h after birth; however treatments were withheld until diarrhoea, indicated by a faecal score of ≥ 2, had developed. This faecal score was generally observed within 2h of the onset of diarrhoea in untreated animals, and generally occurred between 12h and 18h after challenge. Piglets were given a 20ml milk feed immediately prior to the challenge dose, and similar subsequent feeds were given at approximately 2.5h intervals. Piglets were inspected approximately hourly. Where necessary rectal swabs were taken to facilitate reading faecal scores.

Once treatments were commenced they replaced milk feeds. The regime of six treatments was thus given over a shorter period than in the previous experiment (about 15h, rather than 36h). A second challenge dose was not given.

Piglet Management.

In this experiment 21 piglets born to two sows were assessed. Piglet numbers for each treatment were: Control, 5; Ab, 4; Res, 2; prob, 4; ResProb, 2; AbProb, 4. Piglets were housed as described for Example 1. Probiotic-treated animals were again segregated and treated using separate equipment, and appropriate care was used to minimise the likelihood of cross-contamination. Piglets were killed approximately 1h after their 6th treatment.

Faecal and Condition scores/Microbiological Assessments

These were all conducted as previously described. Bacterial numbers in the intestines of piglets treated following onset of diarrhoea are shown in Tables 9 - 12.

Table 9. Mean Hly$^+$ *E.coli* numbers (log_{10} transformed) in the GIT of infected then treated piglets.

Treatment	Stomach	SI - 1	SI - 2	SI - 3
Control	6.200	7.037	7.125	9.134
Ab	6.372	6.547	6.029	8.505
Res	5.397	4.965	6.458	8.148
Prob	6.150	7.342	7.400	8.747
AbProb	5.537	4.899	5.281	7.104
ResProb	4.846	4.020	4.326	7.341

Table 10. Mean lactobacillus numbers (log_{10} transformed) in the GIT of infected then treated piglets.

Treatment	Stomach	SI - 1	SI - 2	SI - 3
Control	5.287	5.913	4.834	4.836
Ab	6.062	5.258	4.609	4.833
Res	6.365	6.974	6.003	6.53
Prob	7.397	6.812	5.466	6.194
AbProb	6.401	6.330	6.858	6.331
ResProb	7.011	6.491	7.272	6.646

Table 11. Mean Coliform numbers (log_{10} transformed) in the GIT of infected then treated piglets.

Treatment	Stomach	SI - 1	SI - 2	SI - 3
Control	5.732	7.214	7.560	9.314
Ab	6.255	6.540	5.879	8.452
Res	5.522	5.073	6.739	8.773
Prob	6.462	8.738	8.161	9.133
AbProb	4.957	4.893	5.386	7.493
ResProb	5.105	4.757	4.374	6.969

Table 12. Mean bacterial numbers (log_{10} transformed) over all GIT sites.

Treatment	Hly$^+$ *E.coli*	Lactobacillus	Coliform	Total Aerobe
Control	7.374	5.218	7.608	7.577
Ab	6.863	5.189	6.871	7.164
Res	6.242	6.468	6.527	6.548
Prob	7.392	6.407	8.240	7.848
AbProb	5.133	6.855	5.301	6.144
ResProb	5.701	6.482	5.705	6.230

Colonisation of the intestines by lactobacilli in piglets infected by *E.coli* at commencement of treatment was comparable at *post mortem* examination to that found in piglets where the treatments were used therapeutically. Generally piglets with higher levels of colonisation were healthier, with the exception of piglets treated with probiotic alone.

Probiotic treatment alone did not appear to be able to reduce colonisation of a pre-existing pathogen population, however, numbers of lactobacilli (Rogosa count), like the total aerobe numbers, may have been encouraged by debilitation of the piglets with diarrhoeal disease.

The combined antibody/probiotic treatments were quite effective in reducing pathogen counts throughout the intestine below colonisation levels generally associated with disease ($10^8 cm^{-1}$ in the lower SI, $10^7 cm^{-1}$ in the mid SI). Combined therapies were clearly more effective than either treatment component alone and because of the higher pathogen numbers in the intestines of the probiotic only piglets, true synergism was evident in the combined therapy. This benefit is exemplified in the ratio of Hly^+:lactobacilli (Table 13) and condition and faecal scores at post mortem (T) and eight hours prior to post mortem (T-8h), (Tables 14 and 15).

Table 13. Ratio of log_{10} transformed Hly^+ *E.coli*:lactobacillus numbers in the GIT's of infected then treated piglets.

Treatment	Stomach	SI - 1	SI - 2	SI - 3	Overall
Control	1.173	1.254	1.474	1.889	1.448
Ab	1.051	1.245	1.308	1.760	1.341
Res	0.848	0.712	1.076	1.248	0.971
Prob	0.831	1.078	1.354	1.312	1.169
AbProb	0.865	0.774	0.770	1.122	0.883
ResProb	0.691	0.619	0.595	1.105	0.753

Table 14. Mean condition scores of piglets at *post mortem* examination (T) and 8h prior to death (T-8h)

Treatment	Mean condition score T	Mean condition score T-8h
Control	2.5	1.7
Ab	1.8	1.3
Res	2.25	2.0
Prob	1.75	1.2
ResProb	1.1	2.6
AbProb	0.5	0.7

Table 15. Mean faecal scores of piglets at *post mortem* examination and 8h prior to death (T-8h).

Treatment	Mean faecal score T	Mean faecal score T-8h
Control	2.6	1.95
Ab	2.8	2.3
Res	2.1	2.1
Prob	2	1.75
ResProb	1.55	2
AbProb	1.0	1.0

Example 3. Production of a composition for use in the invention

Purified clostral antibody is derived by hyper immunization of dairy cows with a protective immunogen from the target pathogen. Immediately after parturition cows are milked twice daily until about 10-20 l of colostrum have been collected from each cow. This colostrum is stored frozen until required for processing. Colostral antibody is then prepared by use of a commercial separator or centrifuge to remove the fat component. The skim colostrum is then treated by ammonium sulphate precipitation and dialysis against distilled water to obtain a fraction containing principally IgG (Fang, W. D. and Mukkur, T.K.S. Biochem J., 1976 155 25). Alternatively, affinity chromatography or other purification techniques could be employed to obtain a desired level of antibody purification. If payload (weight of antibody required for a suitable dose volume) or palatability/physical appearance is not a problem (e.g. treating larger animals such as calves), skim colostrum could be used directly. When treating gastrointestinal diseases with colostral antibody it is generally desirable to remove the fat. Additional purification to a predominantly antibody fraction helps

remove undesirable smells and blood and cellular contamination that may be associated with peripartum lactation. The antibody is conveniently stored and processed in a dry form, following freeze drying or spray drying at low temperature, for example, in a multi-stage drier.

In the present example for treating *E.coli* diarrhoea neonatal in piglets, purified colostral antibody at a dose rate shown to be effective in treating the disease syndrome (such as approximately 12.5mg/kg body weight in this case) is admixed with an appropriate probiotic, also at a dose rate shown effective (such as approximately 10^9 cfu lactobacillus fermentum strain 104/ 100mg tablet) and following addition of appropriate fillers and binding agents, compressed into tablet form. As tablet administration is difficult in neonatal animals, these tablets are preferably dissolved in warm water or milk, prior to dosing with a feeding bottle or by orogastric tube.

Similarly, a tablet for adult animal or human treatment may optionally include traditional enteric coating technologies or a buffered liquid delivery to improve protein or microbiological persistence, in the gastric environment.

Delivery is obviously not limited to combined treatment via tablet, but may be separate tablets or powders separate or combined capsules, granules (macro or micro) or gel formulations.

CLAIMS:

1. A method of treatment or prophylaxis of disease in an animal, said method comprising administering effective amounts of substantially whole antibody and one or more strains of suitable probiotic organisms to said animal, wherein said animal is not naturally protected by exposure to appropriate or adequate antibody.

2. A method according to Claim 1 in which the disease is gastroenteritis or diarrhoea.

3. A method according to Claim 1 in which the gastrointestinal disease is caused by an organism selected from the group consisting of *Escherichia coli*, *Clostridium difficile*, *Helicobacter pylori*, *Cryptosporidium* and rotavirus species.

4. A method according to any one of the preceding claims in which the antibody is derived from a source selected from the group consisting of immune serum, immune colostrum, a monoclonal antibody, and a bioengineered antibody.

5. A method according to Claim 4 in which the antibody is derived from colostrum of an immunized dairy animal.

6. A method according to Claim 5 in which the antibody is bovine IgG_1.

7. A method according to Claim 1 in which the probiotic organism is a *Lactobacillus*, *Bacillus*, *Streptococcus*, *Bifidobacterium* or *Enterococcus*.

8. A method according to any one of the preceding claims, in which the animal is a neonatal human, piglet, calf, foal, lamb, goat or bird, and the gastrointestinal disease is a diarrhoeal disease.

9. A method according to any one of Claims 1 to 7 wherein the mammal is a human selected from the group consisting of immunocompromised patients, patients particularly prone to infection, patients suffering from gastrointestinal malabsorption syndrome, patients undergoing treatment with H_2-receptor antagonists or proton

pump inhibitors, patients suffering from antibiotic-associated diarrhoea, and patients suffering from travellers' diarrhoea.

10. A composition for treatment or prevention of gastrointestinal disease in a mammal or bird, comprising

a) an effective amount of a substantially whole antibody and

b) an effective amount of one or more suitable strains of suitable probiotic organisms,

together with a pharmaceutically-acceptable carrier, wherein said antibody has specificity against an organism or toxin capable of causing gastrointestinal disease.

11. A composition according to Claim 10 comprising a two-part format, in which the antibody is enteric coated or buffered, or is suspended in a buffered liquid excipient either separately or together with the probiotic.

12. A composition according to Claim 11, comprising a multi-component tablet, said tablet optionally being enteric coated.

13. A composition according to Claim 12, wherein the probiotic organism is administered separately from the antibody.

14. A composition according to Claim 13 which is adapted for addition to an animal feed preparation or water.

15. A composition according to Claim 14 which is adapted for addition to an infant food composition.

16. A composition according to Claim 15 which is adapted for addition to poultry feed or water.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/AU 96/00786

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 39/395, 39/40, 39/42, 35/66, 35/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K and Keywords as below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU : A61K and keywords as below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Derwent, Chemical Abstracts : (Lactobacillus, Bacillus, Streptococcus, Bifidobacterium, Enterococcus) and (antibody, immune ( ) serum, immune ( ) colostrum, bovine ( ) Ig, Resus).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	AU, 51577/90 B (628493) (Grahn and Holm) 23 August 1990	1, 7-11
X	AU, 29205/92 A (Reid and Bruce) 27 May 1993	1, 3, 7-11
X	AU, 74657/94 A (Zeneca Limited) 16 March 1995. See entire docment.	1, 4-11

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 March 1997	13 MAR 1997
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer A. WILCOX Telephone No.: (06) 283 2243

INTERNATIONAL SEARCH REPORT

Internatuonal Application No.
PCT/AU 96/00786

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AU, 52547/90 A (Chugai Seiyaku Kabushiki Kaisha; Kyodo Milk Industry Corporatuon Ltd) 11 October 1990 Claims 8, 9 and 19 and Examples 5 and 6.	1-16
P, X	AU 26139/95 A (Dibra S.P.A.) 7 December 1995	1, 4-11
X	AU 41588/93 A (Societe Des Products Nestle S. A.) 13 January 1994 Claims 4, 10 and Examples.	1-16
X	AU 38364/89 A (Whitecliffe Laboratories Limited) 14 December 1989 See entire document	1-16
X	AU 37365/93 A (United States Department of Agriculture) 17 February 1994 See page 9 and claims	1-16
X	AU 82406/87 (624067), B (Biorem C. C.) 16 June 1988 See claims.	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internauonal Application No.
PCT/AU 96/00786

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	51577/90	CA	2047189	CN	1046099	DE	69028520
		EP	458855	FI	913845	HU	57051
		IL	93242	NO	913162	NZ	232381
		PT	93141	SE	8900510	US	5378459
		WO	9009186	ZA	9000692		
AU	29205/92	EP	613374	GB	9124335	GB	2261372
		WO	9309793	WO	9602467	DE	9411327
AU	74657/94	WO	9507090	GB	9318439		
AU	52547/90	CA	2013941	EP	391416	JP	3218318
		JP	3072432	JP	6033386	BR	9305171
		CA	2111921	FI	935782	WO	9629394
AU	37365/93	WO	9612414	ZA	9508786		
AU	26139/95	IT	94501773	NO	964966	WO	9533046
AU	41588/93	CA	2099855	EP	577903	FI	933001
		HU	9301884	HU	66632	JP	6098782
		NO	932407	NZ	248056	US	5578302
		WO	9615475				
AU	38364/89	GB	8827618	GB	8818271	WO	8911858
AU	82406/87	DE	3781652	EP	271364	ZA	8709287

END OF ANNEX